THE METABOLISM OF ISOBUTYRIC ACID LABELED WITH CARBON-13 AND CARBON-14

BY

DONALD SHERMAN WIGGANS

B. S., University of Nebraska, 1949

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
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LEIDEN

I. THE METABOLISM OF ISOBUTYRIC ACID LABELED WITH CARBON-13 AND CARBON-14

Introduction

The metabolic pathway by which valine is metabolized in the intact animal is believed to lead through isobutyric acid as an intermediate. Both valine and isobutyric acid are capable of contributing three of their carbons to the formation of extra glucose in phlorhizin glycosuria (1,2). The present investigation was designed to obtain further evidence as to the fate of the carbon chain of isobutyric acid in the organism of the rat. It seemed particularly desirable to determine which three carbons remain intact in the formation of body constituents. For that purpose, sodium isobutyrate labeled with C^{13} in the methyl groups and C^{14} in the carboxyl group was synthesized and administered to normal adult rats by means of subcutaneous injection. The ratio of the isotopes in glycogen, in various amino acids, and in the excreta showed which of the three carbons are involved.

Experimental

In order to obtain the maximum amount of labeled material, two preparations were carried out. The first gave rise to sodium isobutyrate-3, 3′-C^{13}, and was accomplished by means of a malonic ester synthesis with methyl iodide-C^{13} followed by decarboxylation. The carboxy-labeled salt was prepared with a Grignard reaction using isopropyl bromide and carbon dioxide-C^{14}. A suitable mixture of these two preparations of isobutyric acid was used for injection in the animals.

The subjects in the experiment received approximately 240 microcuries of the C^{14} material and 0.0277 gm. of excess C^{13}. The expired carbon dioxide was collected in sodium hydroxide traps and precipitated as barium carbonate. Glycogen was obtained from a trichloroacetic acid extract of the liver. Tyrosine, cystine, and glutamic acid were isolated from the carcass protein by the use of chromatographic separations. All isotopic determinations were made on carbon

dioxide by means of a mass spectrometer and a vibrating reed electrometer.

Summary

Sodium isobutyrate-1-C^{14}-3, 3′-C^{13} was administered to normal adult rats. The more rapid excretion of C^{14} in the expired air points to the probability that the carboxyl carbon is metabolized more rapidly than the carbon of the methyl groups. After 24 hours, the animals were sacrificed and used for the isolation of glycogen of the liver, and glutamic acid, cystine, and tyrosine of the carcass. The findings demonstrate that glycogen and glutamic acid each contained approximately 20 times as much C^{13} as C^{14}. This provides convincing evidence for the fact that the metabolism of isobutryric acid leads to the production of a three-carbon compound derived largely from the isopropyl group, which is later converted to glycogen and glutamic acid. The presence of small amounts of C^{14} may be accounted for by carbon dioxide fixation.

Tyrosine, as was to be expected, was devoid of both isotopes. The results with cystine are of questionable significance since the quantities of isotopes present in this amino acid were scarcely within the range of accuracy of the analytical techniques. Doubtless, the low content of isotope is to be accounted for by dilution effects incidental to the synthesis of cystine from the three-carbon intermediate.

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II. THE NUTRITIVE VALUE OF PHENYLSERINE

Introduction

It has been shown previously in this laboratory (1) that phenylserine exhibits a marked toxicity for growing rats However, phenylalanine can reverse this effect. The present investigation has confirmed the toxic nature of phenylserine when substituted for phenylalanine in a diet containing a mixture of highly purified amino acids, and has established the precise level of phenylalanine required to reverse the growth inhibition.

Experimental

Phenylserine was prepared benzaldehyde from and glycine by the method of Erlenmeyer and Früstück (2). Four groups of ten animals each were used for the quantitative phase of the study. Each diet contained an amount of DL-phenylserine which was equimolar to the required amount of DL-phenyl alanine in the normal diet. In addition, the diets carried levels of phenylalanine which were 1, 2, 3, and 4 times the amount (1.2 per cent) ordinarily included in a standard amino acid mixture (3). It was found that normal growth occurs when the diets contained the three higher levels of phenylalanine. A significantly lower growth response was noted when a 1 to 1 molar relationship of phenylalanine to phenylserine was employed, although this level of phenylalanine is adequate in the absence of phenylserine.

Summary

Phenylserine has been synthesized and administered to rats as a component of diets containing mixtures of highly purified amino acids. The results demonstrate that phenylserine cannot replace phenylalanine as an essential dietary constituent. Instead, a toxicity is exerted which is reversed by large levels of phenylalanine. The ratio of phenylalanine to phenylserine needed to counteract the toxic symptoms has been established.

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VITA

The author was born on July 14, 1925, in Lincoln, Nebraska. He received his high school training at Jackson and Northeast High Schools in that city. After receiving his diploma from the latter school in 1943, he attended the University of Nebraska for the summer session. In September, 1943, he was inducted into the Army of the United States in which he served for two years and four months. He re-entered the University of Nebraska in February, 1946, and received a Bachelor of Science degree from that institution in June, 1949. In the fall of that year, he enrolled in the Graduate College of the University of Illinois and held a Research Assistantship in the Chemistry Department for two years followed by an Atomic Energy Commission Predoctoral Fellowship from September, 1951, until October, 1952. On June 9, 1951, he married Barbara Ellen Tarrant of Rochester, New York.